

## A genome-wide RNAi screen in *Drosophila* identifies novel regulators of the Ca<sup>2+</sup> / calcineurin/ NFAT signalling pathway

Yousang Gwack, Sonia Sharma, Julie Nardone, Bogdan Tanasa, Alina Iuga, Sonal Srikanth, Heidi Okamura, Diana Bolton, Patrick G. Hogan, Stefan Feske, Anjana Rao  
Harvard Medical School and the CBR Institute for Biomedical Research,  
Boston, Massachusetts

The NFAT family of Ca<sup>2+</sup>-regulated transcription factors has a critical role in vertebrate development and function. In resting cells, NFAT proteins are heavily phosphorylated and reside in the cytoplasm; upon stimulation they are dephosphorylated by the calmodulin-dependent phosphatase calcineurin and translocate to the nucleus. NFAT proteins are not represented in invertebrates, but the pathways regulating their subcellular localization -- Ca<sup>2+</sup> homeostasis, Ca<sup>2+</sup> influx, calcineurin and NFAT kinases -- are strongly conserved across species. Using a genome-wide RNAi screen in *Drosophila*, we have identified several previously unsuspected modulators of NFAT function: *Drosophila* and human STIM proteins which are powerful regulators of Ca<sup>2+</sup> influx in response to depletion of Ca<sup>2+</sup> stores; DYRK, a kinase that directly phosphorylates a conserved motif in the NFAT regulatory domain and deactivates NFAT; and other candidates that affect diverse aspects of Ca<sup>2+</sup> signalling and NFAT regulation. Thus genome-wide RNAi screening in *Drosophila* can be successfully used to cross evolutionary boundaries and identify novel regulators of a transcription factor that is expressed only in vertebrates.

### References

1. Hogan PG, Chen L, Nardone J and Rao A. Transcriptional regulation by calcium, calcineurin and NFAT. *Genes & Dev* 2003; 17: 2205-2232.
2. Feske S, Giltzane G, Dolmetsch R, Standt L, Rao A, Gene regulation mediated by calcium signals in T lymphocytes, *Nature Immunol* 2001; 2: 316-324; Feske S, Prakriya M, Rao A, Lewis RS, A severe defect in CRAC Ca<sup>2+</sup> channel activation and altered K<sup>+</sup> channel gating in T cells from immunodeficient patients, *J Exp Med* 2005; 202: 651-662.
3. Okamura H, Aramburu J, Garcia-Rodriguez C, Viola JPB, Raghavan A, Zhang X, Qin J, Hogan PG, Rao, A, Concerted dephosphorylation of the transcription factor NFAT1 induces a conformational switch that regulates transcriptional activity, *Mol Cell*, 2000; 6: 539-550; Okamura H, Garcia-Rodriguez C, Martinson H, Qin J, Virshup DM, Rao A, A conserved docking motif for CK1 binding controls the nuclear localization of NFAT, *Mol Cell Biol* 2004; 24: 4184-4195.